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Corynebacterium glutamicum as a model bacterium for the bioremediation of arsenic

Summary. Arsenic is an extremely toxic metalloid that, when present in high concentrations, severely threatens the biota and human health. Arsenic contamination of soil, water, and air is a global growing environmental problem due to leaching from geological formations, the burning of fossil fuels, wastes generated by the gold mining industry present in uncontrolled landfills, and improper agriculture or medical uses. Unlike organic contaminants, which are degraded into harmless chemical species, metals and metalloids cannot be destroyed, but they can be immobilized or transformed into less toxic forms. The ubiquity of arsenic in the environment has led to the evolution in microbes of arsenic defense mechanisms. The most common of these mechanisms is based on the presence of the arsenic resistance operon (*ars*), which codes for: (i) a regulatory protein, ArsR; (ii) an arsenite permease, ArsB; and (iii) an enzyme involved in arsenate reduction, ArsC. *Corynebacterium glutamicum*, which is used for the industrial production of amino acids and nucleotides, is one of the most arsenic-resistant microorganisms described to date (up to 12 mM arsenite and >400 mM arseniate). Analysis of the *C. glutamicum* genome revealed the presence of two complete *ars* operons (*ars1* and *ars2*) comprising the typical three-gene structure *arsRBC*, with an extra *arsC1'* located downstream from *arsC1* (*ars1* operon), and two orphan genes (*arsB3* and *arsC4*). The involvement of both *ars* operons in arsenic resistance in *C. glutamicum* was confirmed by disruption and amplification of those genes. The strains obtained were resistant to up to 60 mM arsenite, one of the highest levels of bacterial resistance to arsenite so far described. Using tools for the genetic manipulation of *C. glutamicum* that were developed in our laboratory, we are attempting to obtain *C. glutamicum* mutant strains able to remove arsenic from contaminated water. [Int Microbiol 2006; 9(3):207-215]

Key words: *Corynebacterium glutamicum* · arsenic · biorremediation · leaching · toxic metalloids

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Arsenic: chemical properties and uses

The metalloid arsenic (As) is a member of group V of the periodic table of elements and is thus classified as a heavy metal [42]. Arsenic occurs in nature in four oxidation states (+5, +3, 0, and -3), with pentavalent arsenate [+5, As(V)], and trivalent arsenite [+3, As(III)] being the most common

forms. Elemental arsenic occurs only rarely in nature, but it can be found as microcrystalline masses in Siberia, Germany, France, Italy, Romania, and the USA. Most arsenic is found in conjunction with sulfur in minerals such as arsenopyrite (FeAsS), realgar (AsS), orpiment (As₂S₃), and enargite (Cu₃AsS₄).

Arsenic enters water, air, and land through wind-blown dust and water run-off. In most cases, arsenic occurs natural-

ly within subsurface aquifers. This mobilization of arsenic into the aqueous phase serves as the first crucial step in eventual human arsenicosis [25]. Arsenic in the atmosphere comes from various sources, such as volcanoes (gas fumes), microorganisms, and human release (mainly by burning fossil fuels). Under anoxic conditions, arsenite can be reduced by microorganisms in soil to the volatile compounds arsine (AsH_3) and methylarsines, these compounds being the most toxic forms of arsenic [6].

Arsenic cannot be mobilized easily when in the combined form but, due to human activities, arsenic is emitted as a by-product of copper, lead, and zinc ore refining, and of gold-producing industries [5]. As a consequence of human interference, the arsenic cycle has broadened and large amounts of arsenic end up in the environment and in living organisms [19]. About 90% of all arsenic compounds produced by humans are used as wood preservatives (e.g., for pressure-treated lumber). Treated wood contains chromated copper arsenate (CCA), the most commonly used wood preservative [11]. The remaining 10% is used in insecticides, fertilizers, weed killers, fungicides, glass production, semiconductors, and the production of metal alloys (e.g., in lead-acid car batteries); it is also used as an ingredient in drugs for the treatment of some diseases (e.g., sleeping sickness and chronic myeloid leukemia).

Effects of arsenic in organisms

Arsenic, like other heavy metals, cannot be destroyed once it has entered the environment [40]; thus, arsenic (natural or industrially released) can spread and cause harmful health effects to humans, animals, and other organisms in many locations on Earth. Plants absorb arsenic fairly easily, so that high concentrations may be present in food. Arsenate as inorganic arsenic may be dangerous to human health [4]. It is taken up by marine organisms and enters the food chain as organoarsenic compounds (arsenobetaine). Humans may be also exposed to arsenic through soil, food, water, and air. In those regions of the world where there are natural formations of arsenic, such as the western and southwestern United States, India, and Bangladesh, drinking water may have relatively high arsenic concentrations. Exposure to inorganic arsenic can cause various adverse health effects, including irritation of the stomach and intestines, decreased production of red and white blood cells, skin changes, and lung irritation. It has been suggested that the uptake of significant amounts of inorganic arsenic increases the risk of developing cancer [7,44], especially of the skin, lung, and liver, and lymphatic cancer. Exposures to high concentrations of inorganic arsenic

can cause infertility and miscarriages in women, skin disturbances, declining resistance to infections, brain damage, and cardiovascular effects, including hypertension, coronary artery disease, peripheral vascular disease, and atherosclerosis. Several studies indicate a link between arsenic exposure and type II diabetes [43].

The toxicity of arsenite is due to the formation of strong bonds with functional groups, such as the thiols of cysteine residues and the imidazolium nitrogens of histidine residues from cellular proteins. In the case of arsenate, its toxicity is the result of the mimetic effect of arsenate (AsO_4^{3-}) and phosphate (PO_4^{3-}), which affects global cell metabolism [37].

Arsenic transformation by bacteria

Many types of As transformations have been documented in a variety of microorganisms; those currently seen to lead arsenic speciation in nature are related to oxidations or reduction reactions [24]. These redox reactions are generally carried out by microorganisms either for detoxification or for energy generation to support cellular growth. In some occasions it can be even difficult to discriminate one from the other.

Arsenite oxidation: detoxification and energy generation. Initial reports of microbial As(III) oxidation concerned heterotrophs, and thus As(III) oxidation to generate As(V) in these organisms was viewed as a detoxification strategy (Fig. 1A). This is due to the fact that As(V) is less toxic than As(III), which has a strong affinity for protein thiol-sulphydryl groups. The structural genes coding for arsenite oxidase were recently cloned from *Cenibacterium arsenoxidans* and *Alcaligenes faecalis* and named *aox* and *aso*, respectively [21,36]; *aox* mutants from *C. arsenoxidans* were found to be more sensitive to As(III) than the wild-type parental strain [21]. As(III) oxidation can also enhance heterotrophic growth [HAOs (heterotrophic arsenite-oxidizing bacteria)] or provide the sole source of energy for chemolithoautotrophic growth, as was reported for an *Agrobacterium/Rhizobium*-like organism [32] that used As(III) as sole energy source and CO_2 as carbon source [CAOs (chemoautotrophic arsenite-oxidizing bacteria)]. Recently, the mechanisms involved in the regulation of As(III) oxidation in *Agrobacterium tumefaciens* have been described [10].

Arsenate reduction: energy generation. Dissimilatory As(V) reduction, an anaerobic respiration process that generates As(III) (Fig. 1B), occurs in many

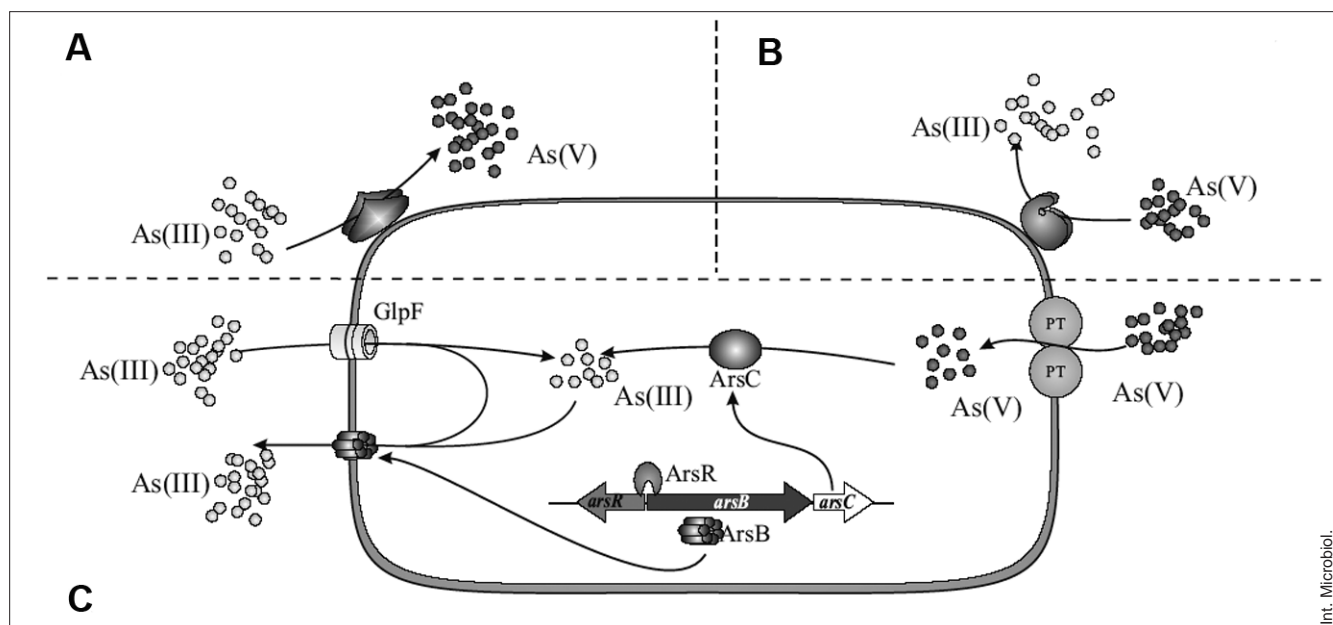


Fig. 1. Diversity of mechanisms present in prokaryotes that oxidize, reduce, or are involved in resistance to arsenic oxyanions. **(A)** Arsenite oxidase is responsible for the oxidation of As(III) by chemoautotrophic (CAOs) or heterotrophic arsenite oxidizer prokaryotes (HAOs). **(B)** Arsenate reductase is involved in the reduction of As(V) by the dissimilatory arsenate-respiring prokaryotes (DARPs). **(C)** Genes and proteins involved in the uptake of As(III) and As(V) (GlpF and phosphate transporters), reduction of As(V) (ArsC), and As(III) extrusion (ArsB) by arsenic-resistant microbes (ARM).

organisms, including *Archaea* and *Bacteria* [DARPs (dissimilatory arsenate-respiring prokaryotes)]. Its discovery was recent [1] and we know far less about the genes required for As(V)-supported anaerobic respiration. Dissimilatory arsenate reductases from bacteria are heterodimeric proteins either located at the periplasm or membrane-associated; their presence in bacteria such as *Chrysiogenes arsenatis*, *Bacillus selenitireducens*, and *Shewanella* has been recently described [36]. Arsenate reductase genes (*arr*) have been well-characterized in *Shewanella*; the *arr* genes conform an operon that is chromosomally joined to the *ars* operon (detoxification), although the two are divergently expressed [30]. However, little is known about regulatory genes and mechanisms involved in the dissimilatory process.

Mechanism of arsenic resistance. The effects of environmental arsenic on human health can be devastating. This aspect, together with the environmental ubiquity of As led to the evolution of arsenic defense mechanisms in every organism studied, from *Escherichia coli* to humans. As described below, organisms take up As(V) via phosphate transporters and As(III) by glyceroporin membrane proteins [19] or hexose transporters [16]. As(V) is then reduced to As(III), which is either extruded from cells or sequestered in intracellular compartments, either as free arsenite or as conjugates with glutathione (GSH) or other thiols. In addition,

arsenic can be methylated, although this process may increase arsenic toxicity and contribute toward detoxification [38]. Detoxification-based As(V) reduction has been best-studied in *E. coli*, and has also been documented for *Staphylococcus*, *Bacillus*, *Acidithiobacillus*, *Pseudomonas*, and a huge group of bacteria [35,36]. This typically occurs in response to As(V) entering the cell via a phosphate transporter. Enzymatic reduction of As(V) to As(III) follows. As(III) is the inducer that triggers up-regulation of the *ars* operon and is also the specific substrate for an efflux pump. As(III) taken up via a glyceroporin will trigger the same set of regulatory responses and thus rapidly extruded from the cell (see Fig. 1C).

Genes involved in arsenic detoxification: the *ars* operon

Resistance to both arsenite and arsenate is widely found among both gram-negative and gram-positive bacteria. Usually, this is in the form of an *ars* operon consisting of a minimum of three co-transcribed genes, *arsR* (determining the regulatory repressor), *arsB* (encoding the membrane arsenite permease pump), and *arsC* (encoding an intracellular arsenate reductase) in the order *arsRBC* (Fig. 1C). Indeed, the *ars* operon occurs more widely in those bacterial

genomes with over 1000 or 2000 genes. It has been argued that arsenite resistance is a very ancient system [19] that must have evolved early after the origin of life because of the wide range of toxic inorganic chemicals that were likely present at that time. The appearance of arsenate-resistant microorganisms, however, is surely more recent; they probably evolved after the atmosphere became oxidizing, which created a pressure for the evolution of an arsenate reductase (ArsC) from a protein-tyrosine phosphatase [20]. The ArsR regulatory protein and ArsB efflux pump protein provide resistance to arsenite, and the full (three elements) *ars* operon confers resistance to arsenite and arsenate.

Occasionally, two additional genes, *arsA* and *arsD*, are found in the *ars* operons of gram-negative bacteria (*arsRDABC*). ArsA is an intracellular ATPase protein that binds as a dimer to the membrane ArsB protein. The arsenite membrane efflux pump is unique in that it can function either chemiosmotically (with ArsB alone) or as an ATPase (with the ArsAB complex). ArsD is thought to be a *trans*-acting co-repressor of the *arsRDABC* operon, in addition to ArsR [35]. In *E. coli* plasmid R733, the *ars* operon comprises the men-

tioned five genes, but in the *Staphylococcus* plasmid pI258, the *ars* operon contains only three genes [35]. A scheme describing the gene organization of the *ars* operons from gram-negative and gram-positive bacteria, with a particular focus on members of the actinobacteria group, is shown in Fig. 2.

Biotechnological interest in corynebacteria and development of molecular biology techniques

Coryneform bacteria belong to the mycolata, a broad and diverse group of gram-positive actinomycetes. Besides a thick peptidoglycan layer, the mycolata contain large amounts of mycolic acids and other lipids in their cell walls [22]. Members of the genera *Corynebacterium* include human and animal pathogens (such as *C. diphtheriae*) and many nonpathogenic members that are of biotechnological importance for the large-scale production of amino acids, such as L-glutamate and L-lysine, and other metabolites [28,29].

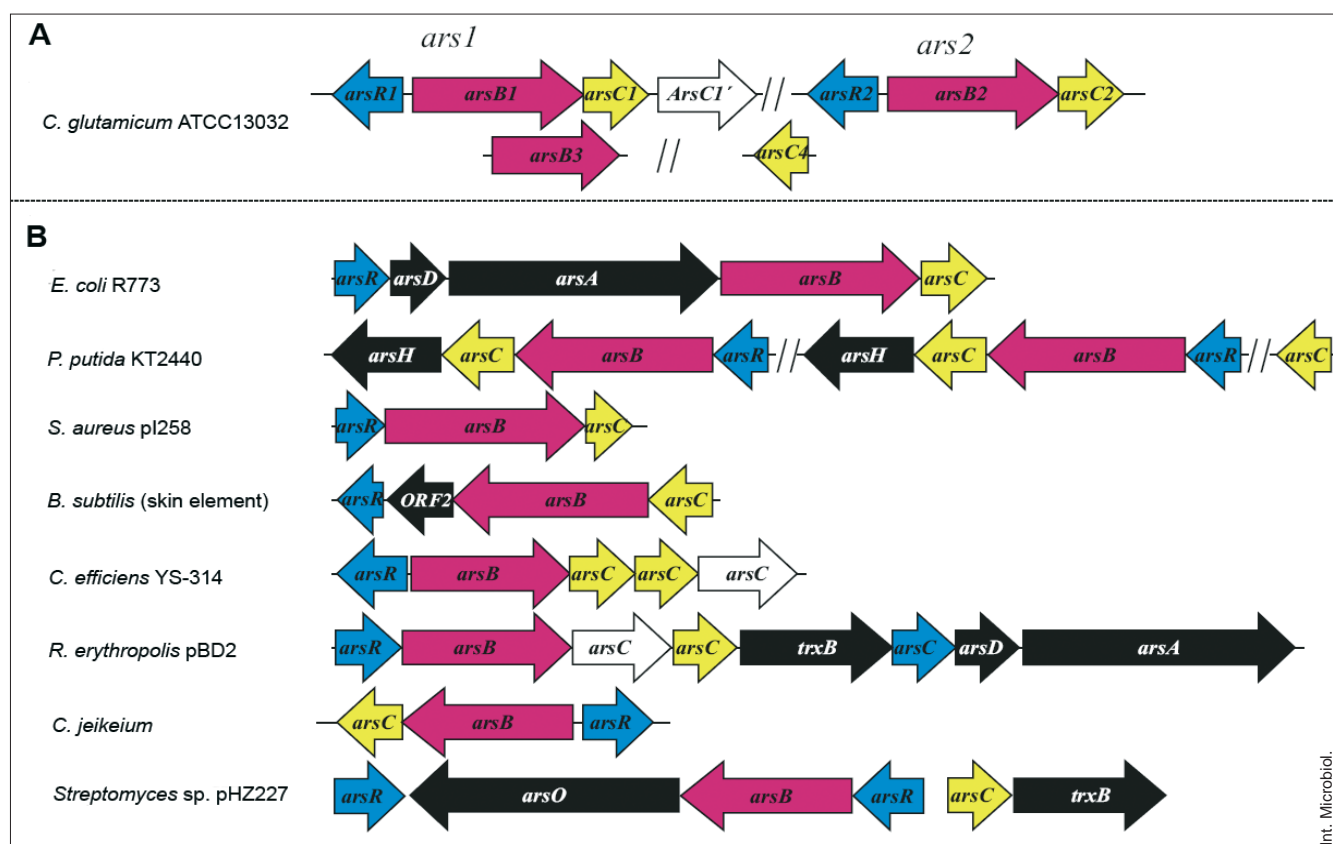


Fig. 2. The genes involved in arsenic resistance in *Corynebacterium glutamicum* (A) and in different microorganisms (B). Arrows represent ORFs found in the sequenced DNAs.

Intrinsic characteristics of members of the coryneform group are: (i) lack of pathogenicity (most of the representatives); (ii) lack of spore-forming ability; (iii) high growth rates; (iv) relatively limited growth requirements; (v) absence of native extracellular protease secretion, and (vi) relative stability of the corynebacterial genome itself. These attributes, combined with an up-to-date set of genetic-engineering tools, make this organism ideal for the development of robust industrial processes that are increasingly competitive with those of *E. coli*, *B. subtilis*, or yeast-based processes. Manipulation of corynebacteria by genetic engineering is not limited to industrial considerations, as corynebacteria also constitute ideal models for understanding the biology of other genera, such as *Gordonia*, *Mycobacterium*, and *Nocardia*, which belong to the same monophyletic taxon. Sequencing of the *C. glutamicum* genome [8,9] marked the dawn of a new era in corynebacterial research; now it is possible to carry out genome-wide genetic modification, which has the potential to generate more efficient and more versatile whole-cell industrial biocatalysts.

Due to the presence of a thick cell wall in *C. glutamicum* and to the absence of any natural or chemically induced DNA uptake system, transformation based on protoplast formation [31] or electrotransformation [2], in addition to transduction and conjugation techniques, have been described in this bacterium throughout the last two decades [12]. Several of the many plasmids endogenous to corynebacteria were adapted for use as cloning and shuttle vectors for the genetic modification of corynebacteria [3,18]. Antibiotic resistance genes have been demonstrated to be useful positive selection markers in corynebacteria, such as genes conferring resistance to kanamycin, chloramphenicol, bleomycin, erythromycin, spectinomycin, and gentamicin [18]. The techniques and tools for genetic engineering in corynebacteria have been discussed in detail in reviews from Kirchner and Tauch [12] and from Vertes et al. [41].

Arsenic resistance in *C. glutamicum*

Resistance to arsenic and genes involved in this process. In view of the ubiquitous presence of arsenic in nature, we assessed the resistance to arsenic of different gram-negative and gram-positive bacteria in complex medium (tryptone soy agar, TSA) supplemented with arsenate [AsO_3^- , As(V)] or arsenite [AsO_2^- , As(III)]. In bacteria, the resistance to As(V) was higher than to As(III). In addition, members of the coryneform group [*C. glutamicum* 13032, *B. lactofermentum* 13869 (presently *C. glutamicum* 13869) and *Rhodococcus fascians* (formerly *Corynebacte-*

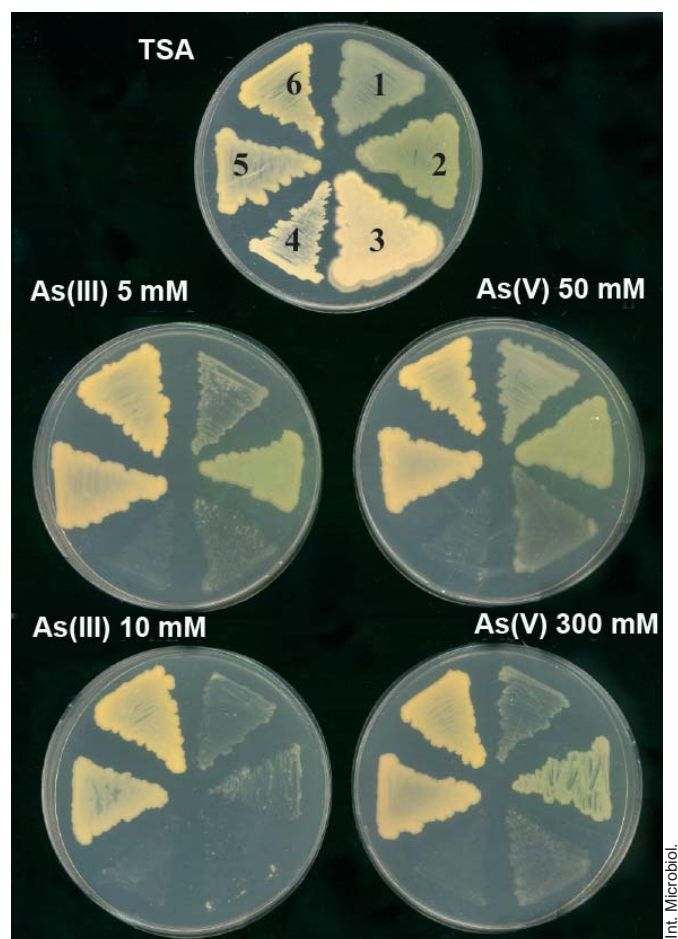


Fig. 3. Resistance level to arsenite [As(III)] or arsenate [As(V)] by *Escherichia coli* DH5 α [1], *Pseudomonas fluorescens* CECT 378 [2], *Bacillus subtilis* CECT 36 [3], *Staphylococcus aureus* CECT 240 [4], *Corynebacterium glutamicum* ATCC 13032 [5], and *C. glutamicum* ATCC 13869 [6].

rium fascians)] were much more resistant to arsenic than the rest of the bacteria analyzed (Fig. 3). In this group, the resistance levels reached for As(V) were close to the solubility level of sodium arsenate in the media (0.4–0.5 M).

The genes involved in arsenic resistance in *C. glutamicum* form two operons, *arsI* and *ars2*, distant from each other on the chromosome [23]. *arsI* and *ars2* have a similar genetic structure that is based on the three-component system (*arsRBC*), with the difference of the presence of an additional gene (*arsC1'*) at the end of the *arsI* operon (Fig. 2A). Genes homologous to *arsC1'* have also been found in *C. efficiens* (accession number: NC_004369) and in plasmid pBD2 from *R. erythropolis* (accession number: NC_005073) (Fig. 2B).

The first genes of the *arsI* and *ars2* operons are *arsR1* and *arsR2*, which encode putative arsenite regulatory proteins. ArsR1 and ArsR2 show clear homologies to ArsR proteins from actinomycetes, but they lack the typical CXCXC

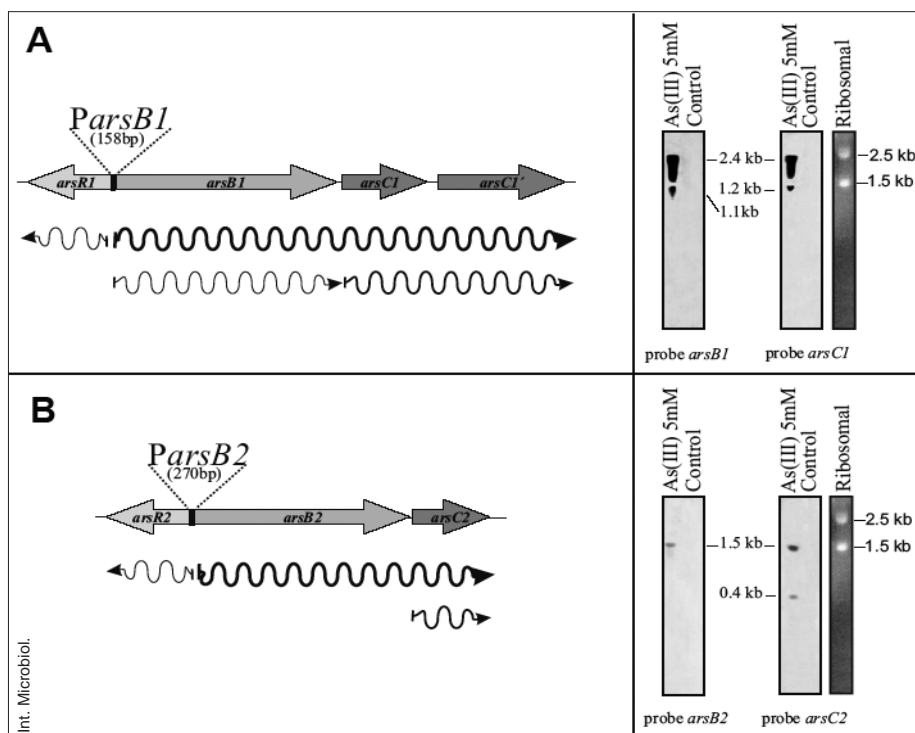


Fig. 4. Specific transcripts for operon *ars1* (A) and *ars2* (B) from *C. glutamicum*. Data were obtained from Northern blot analysis (right) of total RNA isolated from from *Corynebacterium glutamicum* in the absence (control) or presence of 5 mM arsenite. Ribosomal RNAs were used as size controls.

metal-binding motif of many arsenic regulators. Interestingly, *arsR* in both operons is expressed divergently from the rest of the *ars* genes (Fig. 2A). The second genes, *arsB1* and *arsB2*, encode the arsenite efflux pump (arsenite permease). They are homologous to the arsenic protein carriers from actinomycetes, and to the skin element from *B. subtilis* (Fig. 2B). The rest of the genes in both clusters, *arsC1*, *arsC1'* and *arsC2*, encode putative thioredoxin-dependent arsenate reductases with predicted protein tyrosine phosphatase activity. They also share a broad similarity to the arsenate reductases from actinomycetes and, to a lesser extent, with those from *Staphylococcus* [45] (Fig. 2B). In addition to the above-mentioned *ars1* and *ars2* operons involved in arsenic resistance, another putative arsenite permease (*arsB3*) gene and a putative arsenate reductase (*arsC4*) gene are present in the *C. glutamicum* genome (Fig. 2A). *ArsB3* does not show homologies with arsenite permeases from gram-negative bacteria and seems to be different from the *ArsB* clade from actinomycetes; *ArsC4* seems to be unrelated to the other *C. glutamicum* *ArsCs* and shows homologies with *ArsC* from gram-negative bacteria [23].

The involvement of the arsenite permease genes *arsB1*, *arsB2*, and *arsB3* in arsenic resistance in *C. glutamicum* was confirmed using gene-disruption techniques. The resistance levels showed by the single and double mutants obtained suggested that resistance to arsenite [and perhaps also to arsenate through the conversion of As(V) into As(III)] in

C. glutamicum is due mainly to the activity of operons *ars1* and *ars2*, operon *ars1* being more crucial than *ars2*. This was corroborated by cloning the two operons in both *E. coli* (heterologous complementation) and *C. glutamicum* mutant strains. Resistance levels to arsenite for transformed and untransformed *E. coli* and *C. glutamicum* strains were clearly indicative of the effect of the operons in arsenic resistance. In *C. glutamicum* mutants, the presence of *ars1* and *ars2* operons not only complements the respective *arsB1* and *arsB2* mutations, but also confers the strains with hitherto undescribed levels of resistance to arsenite (60 mM versus the original 12 mM) [23].

Transcriptional analysis and regulatory regions of the *C. glutamicum* *ars* operons

Expression analysis of the *ars1* and *ars2* operons revealed that genes *arsB1-C1-C1'* and *arsB2-C2* are transcribed together, and divergently from the transcription of *arsR1* and *arsR2* (Fig. 4A,B). In addition, a clear induction effect by arsenite (5 mM) in the expression of both operons is shown in Fig. 4; Northern analysis revealed that *ars1* was more efficiently expressed than *ars2*. RT-PCR analysis confirmed the expression level of the *ars* operons, but expression of *arsB3* and *arsC4* was very low and constitutive [23]. Thus, *arsC4* was not able to complement the arsenate reductase require-

ments of *C. glutamicum* *arsC* mutants (unpublished results).

Computer analysis showed the presence of a perfect inverted repeat sequence (TGTCGATATT-N₁₂-AATATCGA-CA) 26 nucleotides upstream from *arsB1* and 56 nt upstream from *arsB2* (ATGTCCGTCA-N₈-TGACGcACAT). The promoters located upstream from *arsB1* (*ParsB1*; Fig. 4A) and *arsB2* (*ParsB2*; Fig. 4B) were cloned after PCR amplification and showed promoter activity in *E. coli* (resistance to 200 mg kanamycin/ml) and *C. glutamicum* when adequate promoter probe vectors were used. Both promoters were induced in the presence of arsenite-arsenate ("in vivo") in a range of 0.01–5 mM. However, no induction was observed when different elements or compounds, such as bismuth, antimonite, phosphate, phosphite, nitrates, or nitrites, were assayed. The "in vivo" induction of *ParsB* by arsenite (arsenate) and not by antimonite and bismuth clearly distinguishes these corynebacterial promoters from those of other bacteria [17,27].

Potential use of corynebacteria in bioremediation processes

The main objective of our work with *C. glutamicum* has been to design and construct a corynebacterial strain with special abilities to resist and accumulate arsenic, which can be used for bioremediation. Any strain used for arsenic bioremediation should show increased uptake of arsenate or arsenite

(Fig. 5A) and reduced efflux of arsenite (Fig. 5B). In addition, arsenic present in the cell must be complexed or compartmentalized to enhance cellular tolerance (Fig. 5C).

As mentioned previously, arsenate enters the cell through specific (Pst) or unspecific (Pit) phosphate transporters (Fig. 5A); therefore, the incorporation of arsenate is lower in phosphate-rich media or environments. As(V) uptake increases when *C. glutamicum* is grown in a medium with a low level of phosphate or by reducing the concentration of phosphate by precipitation. In bacteria, arsenite uptake takes place through aquaglyceroporins, but the corresponding genes are absent in the *C. glutamicum* genome (Ordóñez E., unpublished). Arsenite uptake has also been correlated to unspecific sugar transport, such as the hexose permeases described in yeast [16]. Presently, we are looking for genes encoding hexose permeases in the genome of *C. glutamicum* [Villadangos A, unpublished].

To reduce the efflux of arsenite, we disrupted the three arsenite permease genes present in the genome of *C. glutamicum*. One of the double arsenite permease mutants, *C. glutamicum* *ArsB1-B2*, was very sensitive to arsenate and arsenite [23], and was able to accumulate several times more arsenic than the wild-type strain (Ordóñez E., unpublished). We also analyzed the behavior of a mutant with two disrupted arsenate reductase genes (*arsC1* and *arsC2*); in this case, the mutant was highly sensitive to arsenate [unable to grow in the presence of 4 mM As(V)] and, as expected, but no effect on arsenite resistance was observed. The accumulation

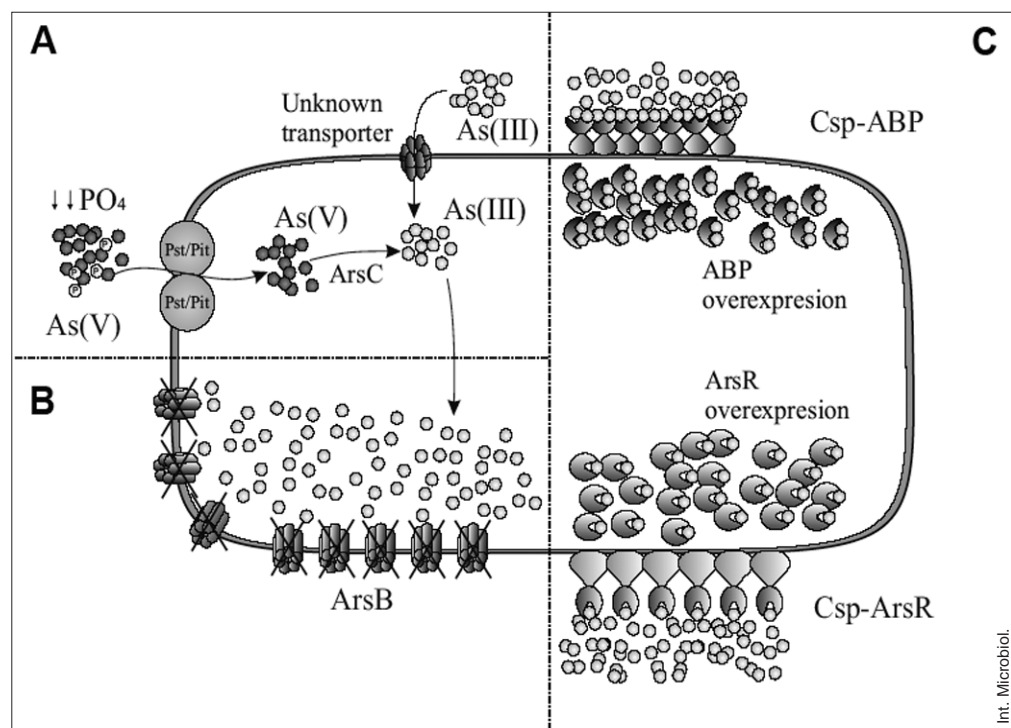


Fig. 5. Different strategies to be used in arsenic detoxification by *C. glutamicum*: (A) increased uptake of As(III) and As(V); (B) genetic disruption of the arsenite efflux pump ArsBs; and (C) biosorption of arsenic inside or outside the cells. Csp, *C. glutamicum* S-layer protein; ABP, putative arsenic binding peptide. (For details, see text.)

of As by these mutants has been studied, and they are being used for arsenic speciation [Feo JC, unpublished results].

Several strategies to remove heavy metals from contaminated environments (bioremediation) have been described. The use of plants for arsenic remediation is the best-known due to the presence of phytochelatins (PC), which are induced in plants in the presence of the toxic agent [34]. This ability has been exploited to increase the cellular content of cadmium and arsenic (20- and 50-fold, respectively) by cloning the PC synthase genes from *Arabidopsis thaliana* in *E. coli* [33]. A specific method to increase the cellular concentration of As was achieved in *E. coli* by cloning the gene encoding ArsR in a multicopy plasmid as a fused protein; the resulting strain was able to accumulate up to 50 ppb of arsenite (100% removal) from contaminated water [13,14]; however, this strain would not be useful to remove higher As levels. Equivalent strains overexpressing PC synthase genes and/or ArsR could be developed in *C. glutamicum* (Fig. 5C).

One of our aims is the construction of *C. glutamicum* strains able to accumulate heavy metals outside the cell (used as biosorbent), as was described previously for *E. coli* [15] and *Ralstonia eutropha* [39]. In both cases, a metal-binding peptide or a gene encoding a mouse metallothionein (MT) was fused to a gene encoding an outer membrane protein, and the fused protein was targeted to the outer membrane. Although *C. glutamicum* does not have an outer membrane, it contains a typical cell-surface S-layer formed by a protein encoded by *cspB* [26]. Our goal is to fuse *cspB* to: (i) the *arsR* gene; (ii) genes involved in MT/PC; or (iii) gene fragments for As-binding peptides (ABP), in order to obtain a *C. glutamicum* strain to be used for arsenic bioremediation of highly contaminated waters (Fig. 5C).

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***Corynebacterium glutamicum* como bacteria modelo para la biorremediaci n de ars nico**

Resumen. El ars nico es un metaloide extremadamente t xico y su presencia a concentraciones elevadas es una amenaza grave para la biota y para la salud humana. La contaminaci n del suelo, agua y aire por ars nico es un problema ambiental que va en aumento en todo el mundo debido a la lixiviaci n de formaciones geol gicas, al uso de combustibles f siles, al vertido incontrolado de residuos de la industria minera del oro, y a los usos inadecuados en agricultura o medicina. A diferencia de los contaminantes org nicos, que se descomponen en compuestos qu micos inofensivos, los metales y los metaloides no se destruyen, pero pueden ser inmovilizados o transformados en formas menos t xicas. La ubicuidad del ars nico en el ambiente origin  la aparici n de mecanismos de defensa en los microorganismos; el mecanismo m s frecuente se basa en la presencia del oper n de resistencia al ars nico (*ars*), que codifica (i) una prote na reguladora, *ArsR*; (ii) una permeasa de arsenito, *ArsB*; y (iii) una enzima que interviene en la reducci n del arsenato, *ArsC*. *Corynebacterium glutamicum*, usado para la producci n industrial de amino cidos y nucle tidos, es uno de los microorganismos m s resistentes a ars nico descritos hasta la fecha (hasta 12 mM de arsenito y >400 mM de arseniato). El an lisis del genoma de *C. glutamicum* ha revelado la presencia de dos operones *ars* completos (*ars1* y *ars2*) que muestran la estructura t pica de tres genes *arsRBC*, con un gen extra *arsC1'* situado en el extremo 3' de *arsC1* (oper n *ars1*), y dos genes hu rfanos, *arsB3* y *arsC4*. La intervenci n de ambos operones *ars* en la resistencia al ars nico en *C. glutamicum* fue confirmada por la interrupci n y amplificaci n de dichos genes. Se obtuvieron cepas resistentes a concentraciones de hasta 60 mM de arsenito, que es uno de los niveles m s altos de resistencia bacteriana a arsenito descritos hasta ahora. Por medio de las t cnicas de manipulaci n gen tica de *C. glutamicum* desarrolladas en nuestro laboratorio, estamos tratando de obtener cepas mutantes de *C. glutamicum* capaces de eliminar ars nico del agua contaminada. [Int Microbiol 2006; 9(3):207-215]

Palabras claves: *Corynebacterium glutamicum* · ars nico · biorremediaci n · lixiviaci n · metaloides t xicos

***Corynebacterium glutamicum* como bact ria modelo para a biorremedia  o de ars nio**

Resumo. O ars nio   um metaloide extremamente t xico e sua presen a em concentra  es elevadas   uma amea a grave para a biota e para a sa de humana. A contamina  o do solo,  gua e ar por ars nio   um problema ambiental que aumenta cada vez mais em todo o mundo devido   lixivia  o de forma   es rochosas,   combust  o dos combust veis f sseis,   libera   o incontrolada de res duos da ind stria de explora   o mineral do ouro, e aos usos inadequados na agricultura ou medicina. Diferentemente dos contaminantes org nicos, que se degradam em compostos qu micos inofensivos, os metais e os metal ides n o se destroem mas podem ser imobilizados ou transformados em formas menos t xicas. A ubiq idade do ars nio no ambiente levou   evolu   o de mecanismos de defesa nos microrganismos; o mecanismo mais comum se baseia na presen a do oper n de resist ncia ao ars nio (*ars*), que codifica (i) uma prote na reguladora *ArsR*, (ii) uma permease arsenita *ArsB*, e (iii) uma enzima que interv m na redu   o do arsenato, *ArsC*. *Corynebacterium glutamicum*, que   utilizado para a produ   o industrial de amino cidos e nucle t dios,   um dos microrganismos mais resistentes ao ars nio descritos at  o momento (12 mM de arsenito e >400 mM de arseniato). A an lise do genoma de *C. glutamicum* revelou a presen a de dois oper ns *ars* completos (*ars1* e *ars2*) que mostram a estrutura t pica de tr s genes *arsRBC*, com um gene extra *arsC1'* situado no extremo 3' de *arsC1* (oper  n *ars1*) e dois genes  rf os *arsB3* e *arsC4*. A interven   o de ambos oper ns *ars* na resist ncia ao ars nio em *C. glutamicum* foi confirmada pela interrup   o e amplific   o dos genes. Se obtiveram cepas resistentes a concentra   es de at  60 mM de arsenito, que   um dos n veis mais altos de resist ncia bacteriana a arsenito descritos at  o momento. Por meio das t cnicas de manipul   o gen tica de *C. glutamicum* desenvolvidas em nosso laborat rio, estamos procurando obter cepas mutantes de *C. glutamicum* capazes de eliminar o ars nio da  gua contaminada. [Int Microbiol 2006; 9(3):207-215]

Palavras-chave: *Corynebacterium glutamicum* · ars nio · biorremedia   o · lixivia   o · metaloides t xicos